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LIFE HISTORIES AND HETEROTHALLISM OF THE RED BREAD-MOLD FUNGI OF THE MONILIA SITOPHILA GROUP

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LIFE HISTORIES AND HETEROTHALLISM OF THE RED BREAD-MOLD FUNGI OF THE MONILIA SITOPHILA GROUP[1]

By C. L. SHEAR, *Senior Pathologist in Charge, Mycology and Disease Survey*, and B. O. DODGE, *Pathologist, Fruit Diseases, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

The red bread mold has long been known as a bakery pest and has caused much loss to bakers as well as to housewives. Its occurrence is somewhat erratic, but when a bakery becomes infested there is more or less continual trouble and loss until the fungus has been thoroughly eradicated.

Related species develop extensively on sugar-cane bagasse and this becomes a serious problem where this bagasse is used commercially as a basis for insulating board. The fungi of this group have also been found on silage and sometimes in connection with field, transportation, and storage rot of fruits, especially strawberries, raspberries, and apples, but appear to be of little economic importance in such cases. They are also well known to laboratory workers in mycology and pathology as a contamination in cultures of other organisms. The conidia are so light and powdery that they are easily and widely disseminated by the faintest breeze or movement of the air to which they are exposed.

HISTORICAL REVIEW

The common red mold appears to have been first reported in scientific literature by Léveillé (*37, p. 5, 6, 8*)[2] in France in 1843 under the name *Oïdium aurantiacum*. The same year it was also described by Montagne (*35, p. 377–378*) as *Penicillium sitophilum*. It was finally transferred to the genus Monilia by Saccardo. The fungus was called to the attention of Léveillé and Montagne by a commission (*37*) which was appointed by the French Minister of War in 1842 to investigate the cause of an infestation of the army bakeries which destroyed large quantities of bread. Another outbreak of the mold in bakeries in France was reported in Paris in 1871 by Decaisne (*9, 10*). De Claubry (*18*) also investigated this case and says that he had seen the same thing in bread in France in 1831. Decaisne states that sickness in one family whose members ate the moldy bread was attributed to the mold, but no evidence was found to support this. Outbreaks of the same fungus in bakeries have been reported by Murtfeldt (*36*) in Germany in 1917, by Mattirolo (*32*) in army bakeries in Italy in 1918, and by Herter (*20*) in Berlin in 1919. The same trouble is frequently reported from bakeries in this country, and only a few weeks ago cultures of the fungus were received from two different bakeries in New York.

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[1] Received for publication Feb. 3, 1927; issued July, 1927.

[2] Reference is made by number (italic) to "Literature cited," p. 1040.

In connection with the investigations of this fungus made in France, L. R. and C. Tulasne (*49, p. 90*) give an interesting account of its temperature relations, which being translated reads as follows:

> Mauduyt of Poitiers, an expert in all branches of the natural sciences, in whose company we received our first knowledge of them, once thought that the dust [spores] of *Oidium aurantiacum* Lév. (*Penicillium sitophilum* Mont. in Ann. Sci. Nat. ser. II, t. xx, 1843, p. 377, pl. 16, f. 4, and Syllog. Pl. Crypt. 1856, p. 301), when mixed with ground meal and then baked in a hot oven, was damaged, so that if the bread afterwards went bad he thought the reason was to be found not at all in the included dust but in spores fallen from the air. He also tested some bread of the previous day which was everywhere much defiled by the *Oidium*, and when he had boiled it in water for half an hour it was never again spoilt by the contained mucedinous fungus or covered afresh by the red powder, but a piece of the same bread, when purified by similar boiling, could be readily infected by the seeds of *Oidium* falling from the air or purposely scattered over it, so that within two days it became completely red. (*Cf. Epist. ad Pictonum ediles* missam, Oct. 30, 1847.) The recent experiments of the famous L. Pasteur on the Mucedinei agree with the statements of the Poitevin naturalist. For they prove that the same spores of Mucedines, which could withstand without harm a heat of 120° or 125° for one hour or more in dry air or in a tube exhausted of air, were killed at once in boiling water; and the same thing happened with the dusty particles floating in the air, to which, falling perpetually, that author thinks all kinds of fermentation are due. (*Cf. Comptes Rendus*, t. LII., p. 16–19, Jan. 7, 1861.) All this agrees with what Turpin says about *Torula Cerevisiae* Turp. (in Walter Claubey's *Repert. Chim.* t. for 1838, p. 272, 279), for that fungus, as shown by Colin and Thénard, when placed for a little while in boiling water, lost at least for a time thereby any power of exciting the fermentation of a sugary liquid. Therefore the mode of action of the Gascons is seen to be contrary to the opinions of the learned, and we believe the only experiments on which reliance can be placed are those by which Payen asserted he had proved that the seeds of *Oidium aurantiacum* Lév., even when moistened retained their power of germinating uninjured, after being subjected to a temperature of 105° and 120°, so that the experimenter need not be surprised if bread made of contaminated wheat soon goes red on account of the Mucedine which it contains, since the inside of the loaf is not thought to be exposed in the oven to a heat of 100°. (*Cf. Comptes Rendus*, t. xxvii, p. 4–5, July 3, 1848.)

The mold in loaves of bread develops from spores contaminating either the flour after it has been milled or the wheat before it is ground. After the mold has been introduced into a bakery the dough from which the bread is made may also be contaminated by spores floating in the air of the bakery. According to recent experiments made by Tokugawa and Emoto (*48*), conidia of *Monilia sitophila* are killed in saturated steam at 100° C., although in a moist condition they are able to survive a temperature between 70° and 80° for five minutes, and in a dry atmosphere they are able to endure a temperature as high as 130°. There would be little probability, according to the statements of the authors just cited, of the conidia being destroyed in the loaf in baking, since records of baking temperatures made by the Bureau of Home Economics of this department show that when bread is baked in an oven at a temperature of 200° C. the temperature of the interior of the loaf does not exceed 95° to 98°. According to the temperature tests of the writers, conidia immersed in water and heated to 72° for four minutes are killed, and most of them are killed when subjected to a temperature of 65° for the same period. Moisture conditions, period of heating, and other factors enter so largely into the determination of the death point that it is difficult to tell whether or not the spores would be killed in ordinary baking.

Various physiological experiments with this mold have shown that it will cause fermentation and produce alcohol in the absence of

oxygen. It has also been demonstrated by Went (*51*, *52*) that it produces 10 different enzymes as follows: Maltoglucase, trehalase, raffinase, invertase, cytase, diastase, lipase, tyrosinase, labenzym, and trypsin. Kunkel (*27*, *28*) made a study of the toxicity of various inorganic salts upon the growth of this fungus. He found that of various nitrates tested potassium nitrate is least toxic and zinc most toxic. He also found that toxicity measurements made without regard to the inorganic substances in the culture medium are of little value.

In none of the literature relating to this fungus that the writers have been able to find is there mention of the finding of perithecia of *Monilia sitophila* either in nature or in artificial cultures. Went (*51*) in his physiological studies describes and illustrates the beginning of sclerotoid bodies resembling young perithecia. His only reference to them, however, is in the explanation of the plate in which he calls them "Hyphenknauel." They may have been the fundaments of sterile perithecia.

In 1901 Alfred Möller (*34*, *p. 294*, *pl. 2*, *fig. 34*), in his studies of tropical fungi, described a red mold found on corn bread and carbonized tree trunks, which he named *Melanospora erythraea*. In growing this fungus in culture he obtained an orange-colored mold which he did not attempt to name, but which according to his description and illustrations taken in connection with the writers' recent studies of the life history *of Monilia sitophila* was undoubtedly very closely related to this species, since the perithecial as well as the conidial forms are very similar. No one seems to have recognized heretofore the close relationship of Möller's conidial fungus to the common *M. sitophila*.

In 1924 Kitasima (*24*, *25*), of Japan, published a report of observations which he had made upon an orange-colored mold found growing abundantly on trunks of trees which were burned during the great fire in Tokyo. He referred the fungus to *Monilia aurea* Gmel. Growing this in culture he obtained perithecia and ascospores of a fungus which he referred to the genus Anthostomella. An examination of Kitasima's fungus, which he sent the writers, shows that his conidial stage can not at present be distinguished from the common *M. sitophila*, and that the perithecial form, which he called Anthostomella, is really more nearly related to Melanospora as generally interpreted, and is evidently the same as that which the writers have obtained from *M. sitophila*.

LIFE HISTORY AND TAXONOMIC STUDIES

A few years ago Charles Thom, of the Bureau of Chemistry, in studying the organisms found on sugar-cane bagasse obtained cultures of a Monilia which produced both conidia and perithecia. Thom and Lathrop (*47*) in referring to this fungus say: "On the surface of the bales the first organism to develop was *Monilia sitophila*, which spread over the piles with great rapidity and draped them with festoons of orange-fruiting masses. Even after three to four weeks, handfuls of mycelium and spores could be gathered in many places." Cultures of this fungus furnished by Thom and Miss V. K. Charles, of the Bureau of Plant Industry, were studied and single ascospore cultures obtained which produced conidia very similar to typical *M. sitophila*.

A little later several cultures labeled *Monilia sitophila* were obtained from Westerdijk's culture collection at Baarn, Holland. One of these had been supplied by Stahel, of Dutch Guiana, another came from Herter, of Berlin, and another was originally grown by Bainier, of France. Much to the surprise of the writers, the culture from Dutch Guiana developed many perithecia, which were very similar in character to those in the cultures from Japan and Louisiana. Careful comparison, however, of perithecia, ascospores, and conidia shows that there are specific differences between them. These differences are shown by the descriptions and illustrations which follow.

A part of Möller's type of *Melanospora erythraea*, sent by H. W. Wollenweber, of Berlin, shows that it is different from the Surinam plant which the writers had at first thought might belong to the same species.

The association in cultures of conidia similar to those of *Monilia sitophila* and perithecia, which were referred by Möller (*34*) to Melanospora and by Kitasima to Anthostomella, naturally suggested a genetic relation between the two spore forms. In fact Kitasima (*25, p. 17*) says: "I have succeeded to get a pure culture derived from a single ascospore, produced the conidia which are of Monilia type in coloration, size, and shape." He does not state that perithecia appeared in this culture. The writers do not find, however, that anyone has reported the production of good perithecia in monosporous cultures of either conidia or ascospores and their early attempts were equally unsuccessful. This led naturally to the suggestion that the writers were dealing with a group of heterothallic species.

First efforts to grow single ascospore cultures were largely unsuccessful, owing to failure of the ascospores to germinate under ordinary conditions. It was soon found, however, that heating the spores caused them to germinate more readily. By pairing a large number of different ascospore or conidial cultures from different sources, it was demonstrated that certain paired cultures produced perithecia in abundance while others produced none. It was thus demonstrated that of the three species with which the writers have worked two are heterothallic, i. e., consist of two sexually different haplonts, and one species is homothallic. A full account of this phase of the work will be given in connection with the discussion of culture studies.

The discovery of another spore form of this mold may have important bearings upon the means of dissemination and the chances of reproduction of the fungus, and hence of its prevention and control. It is also important to know that there are several species which may infest bread, fruit, and other plant products and that they are heterothallic. If only one sex is present, the fungus should be easier to eradicate when it is found in a bakery or any other place, as no ascospores would be produced.

In attempting to determine the identity and systematic relationships of these species it was necessary to compare them carefully with the genera having similar perithecial forms as well as conidial stages. In the present state of our knowledge of the Pyrenomycetes it is difficult to define accurately and to limit the various genera that have been described. As at present treated, many genera include a heterogeneous group of species. It is therefore desirable to select some particular species of a genus as a basis for a concept of the group.

As already mentioned, two of the species with which the writers are concerned have been referred to different genera. Möller (*34*) referred his species to Melanospora, and Kitasima (*24*) referred his to Anthostomella, the genus to which *Melanospora destruens* (Shear) comb. nov. was first referred.

Melanospora was described by Corda (*7, p. 24*) in 1837. He included in it three species in the following order: *Melanospora zamiae, M. chionea,* and *M. leucotricha,* all of which he illustrated. The first species, *M. zamiae,* has not since been reported, as far as known, except for a specimen found on rice in India and so named by E. J. Butler. The other two species are very similar and apparently congeneric. The life history of neither of these is satisfactorily known. Zukal (*53, p. 538*) grew *M. leucotricha* but obtained no conidia or pycnidia. The ascospores germinated from one end only. In accordance with present usage and in the interest of stability and uniformity in nomenclature it seems best to accept *M. chionea* (Fr.) Cda. (pl. 1, A), the commonest and best known species of Corda, as the nomenclatorial type of Melanospora, *M. chionea* has membranous, gray perithecia, with a long slender beak which is fimbriate at the apex. No conidia are known. The perithecial form of *Monilia sitophila* differs from this in the color and character of the perithecia, in the absence of a distinct elongate fimbriate beak, and in its persistent asci.

Anthostomella, to which Kitasima (*25*) referred the perithecial form of his Monilia, is really less nearly related to this fungus than Melanospora and several other closely related genera. Anthostomella was described by Saccardo (*40, p. 84*). *Anthostomella limitata* Sacc., the first of the three species originally placed in the genus by the author, is apparently a good representative of the group as generally accepted. The perithecia in this species are submembranous, gregarious, entirely embedded in the tissue of the host, and covered with a more or less blackened clypeus or epistroma. Good paraphyses are also present and no conidial form is known.

Ceratostoma of Fries (*15, p. 337*) is another related genus described in 1818 with 35 species. *Ceratostoma* (*Melanospora*) *chionea* was included as No. 16 of the list. In 1849 Fries (*16, p. 396*) revised his idea of the genus and included but six species, specifying particularly *C. chionea* as the type of the genus. In a footnote he makes the following statement (translated):

> When I collected *S. chionea* I immediately considered it to be the type of a new genus=Ceratostoma Obs. Myc. II; then I saw a very close relationship to various beaked Sphaerias which could be in no way distinguished. For this reason I reduced them to Sphaeria in the S. M., and moreover the limits are changing, for Sphaerias No. 110–112 should be placed here with equal justice. Meanwhile following the suggestion of recent authors, I separated this and the following subgenera, at the same time predicting a close connection with Sphaeronaema; for species of this genus having a globose perithecium sharply distinct from a beak can not be distinguished from Ceratostoma. The name Melanospora Cord. is suitable for only the first species, therefore, we have fixed upon the older name to be retained.

It is clear from this that he regarded his genus as a synonym of Melanospora of Corda. The usage of later authors, however, as Fuckel (*17, p. 127*), Schroeter (*42, p. 312*), Saccardo (*41, p. 215*), and Lindau (*29, p. 406*) has changed the application of the name by retaining Melanospora for *M. chionea* and similar species, and

applying Ceratostoma to a group of species of which *C. notarisii* Sacc. may be designated as the nomenclatorial type. In adopting this interpretation Lindau (*29, p. 406*) says in a footnote (translated):

> As Schröter has shown, Fries's concept of the genus does not correspond with the present application of the name. The species he recorded as belonging to it must be referred to Melanospora. If one follows priority *Ceratostoma* must replace *Melanospora*, and a new name be given the present group of species. This would only make confusion. I therefore accept the present usage.

According to this interpretation of Ceratostoma the writers' species differ from it in having neither a carbonous, long rostrate perithecium nor a true persistent paraphyses and in having a Monilia conidial stage.

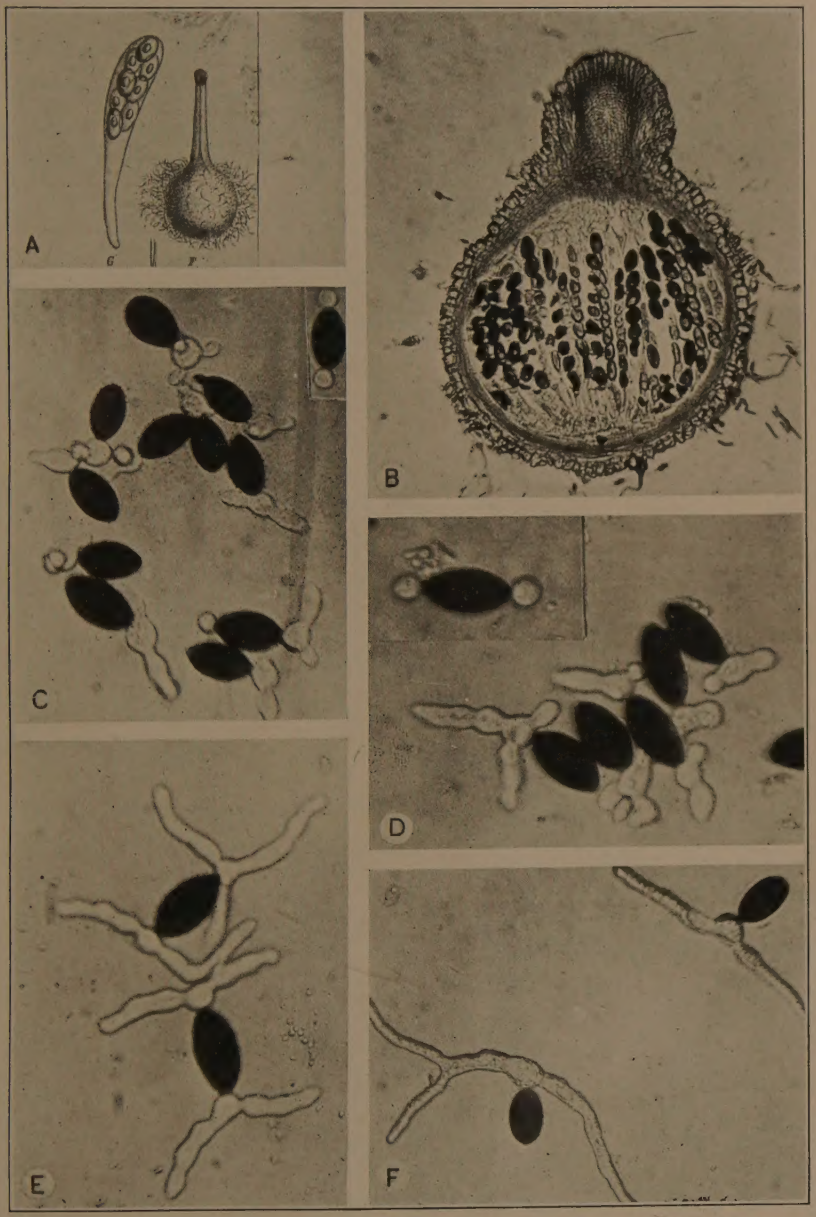
Included in Sordaria and Podospora are certain species which show many points of relationship with the fungi treated here. Some of these species are known to have conidia of the Spicaria type, e. g., *Sordaria montanensis* Griffiths (*19, p. 49, pl. 19, fig. 13*) and *Podospora brassicae* Brefeld (*5, p. 196–198, pl. 6, figs. 1–2*). The same thickened ring is also found in the apex of the ascus in some species of these genera. *Ascotricha pusilla* (E. and E.) Chivers (*6, p. 220, pl. 17*) and some species of Chaetomium also show close relationship.

Certain species referred to Rosellinia, Sphaeroderma, Erythrocarpa and Sphaerodermatella are also nearly related and are in need of further study and comparison before the natural boundaries and relationships of these genera can be determined.

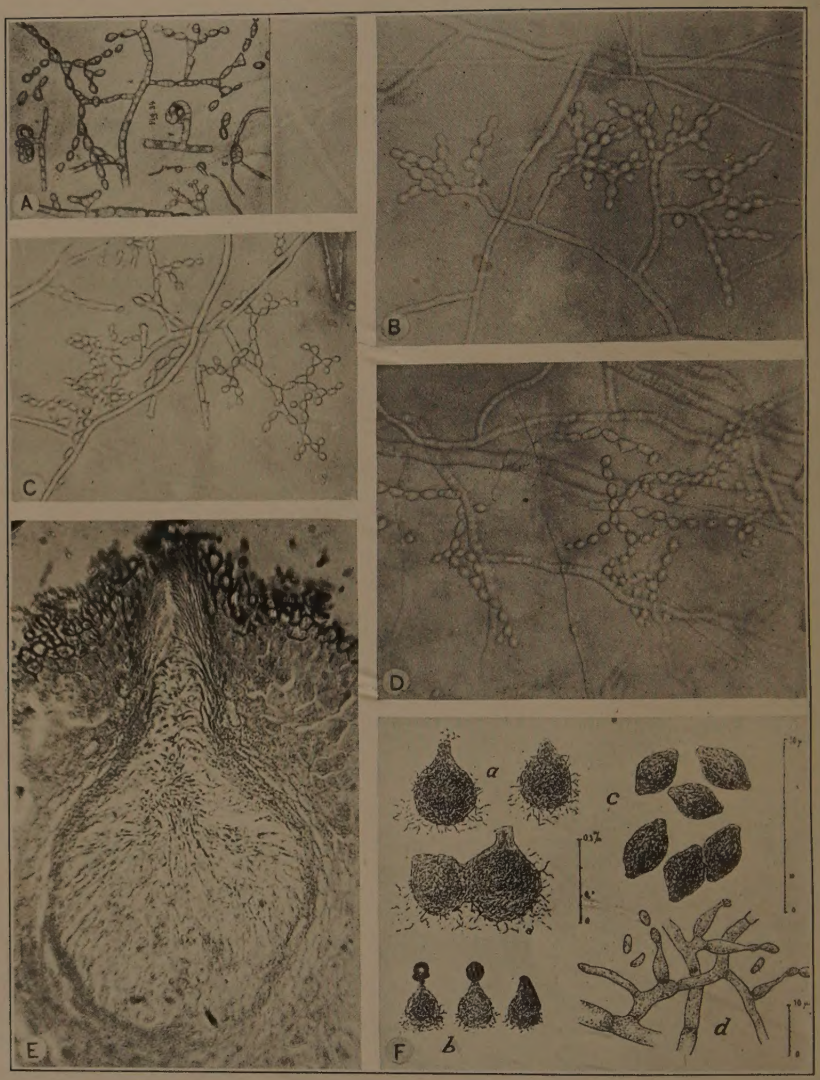
As far as known, the nearest relative of the new genus Neurospora, to which the red bread-mold fungus is being referred, was described by Vincens (*50*) as *Melanospora mangini* (pl. 2, F). This has very similar perithecia and the ascospores have anastomosing ridges. It also has a conidial stage which the author refers to Spicaria (pl. 2, F, *d*). Several other species referred to Melanospora have known conidial forms which are referred to Spicaria. These are evidently more closely related to the new genus Neurospora than to true Melanospora. Whether they should be transferred to this new genus or not must depend upon further knowledge of the systematic value of the ascospore characters and the conidial stage. The conidial forms of other species of Melanospora have been referred to Scopulariopsis and Acmosporium. Another group of species which has been referred to Melanospora may be represented by *M. destruens* (Shear) (*43; 44, p. 43*) (pl. 1, B; pl. 3, D). This group differs from the typical Melanospora as reeprsented by *M. chionea* in having thicker subcoriaceous perithecia and short, nonfimbriate ostioles.

Small globose bodies resembling sclerotia and known as bulbils, which have been found associated with cultures of species referred to Melanospora and are placed in the form genus Papulospora as treated by Hotson (*22*), usually develop in monosporous cultures of the heterothallic species of Neurospora, and sometimes occur in the homothallic species and in the cultures from the paired haplonts. Berkhout (*2, p. 39*) also reports such bodies in her cultures of *Monilia sitophila*. Whether these are the fundaments of sterile or aborted perithecia or a peculiar form of asexual body can not be stated at present.

The genus Monilia, to which the conidial stages of Neurospora are here referred, was first described by Hill (*21, p. 69*) in 1751. In this



Photomicrographs of Melanospora and Neurospora: A, Perithecium and ascus of *Melanospora chionea* (after Winter); B, section of perithecium of *M. destruens;* C–F, germinating ascospores; C. *Neurospora sitophila;* D, *N. crassa;* E, *N. tetrasperma;* F, *Melanospora destruens.* B× 148,C–F × 368



Conidia and perithecia of Neurospora and Melanospora: A, Conidia of *N. erythraea* (after Möller); B, *N. sitophila;* C. *N. crassa;* D, *N. tetrasperma;* E, section of young perithecium of *N. crassa* showing cavity filled with degenerating hyphae resembling paraphyses; F, perithecia, *a, b*; ascospores, *c*, and conidia, *d*, of *Melanospora mangini* (after Vincens). A–D × 185. E × 216

connection it is interesting to note that he makes a statement which it has taken nearly 200 years to verify. His statement is as follows:

> The Monilia all produce distinct male and female flowers. The male flowers are antherae, placed on a short stamina, on the summit of the pedicle, or near the summit, surrounding it in form of a fine powder. The female flowers we are able to distinguish nothing of, except the seeds, which are arranged together in series by means of a glutinous matter, and stand sometimes close to one another, sometimes more distinct.

This was written during the period when the question of sex in flowering plants was being much discussed by botanists. Hill was one of those who having accepted sex in plants thought it should be true of fungi as well as flowers; hence his rather naïve attempt to distinguish male and female flowers in Monilia.

Hill's conception of the genus was based upon Micheli's (*33, p. 212, tab. 91*) descriptions and figures of Botrytis and Aspergillus. The present so-called form genus Monilia is not usually credited to Hill because he was not a binomialist, and because of his broad and too indefinite application of the name.

The name is usually attributed to Persoon (*38, p. 691*), who first used it in 1801 to include 12 species. These were as heterogenous as those of Hill. The original species of Persoon have gradually been separated and most of them placed in other genera. According to present usage, *Monilia fructigena* Pers., the conidial stage of *Sclerotinia fructigena*, might well be taken as the nomenclatorial type of Monilia. If the names of conidial stages were to be restricted to those having the same generic perfect stage it would be necessary to refer *M. sitophila* to another form genus. It does not seem desirable, however, to follow such a course, as the inconvenience and change would be much greater than any benefit which might be derived. Until all the conidial forms have been connected with their perfect stages it will be necessary to retain these form genera to include conidia and pycnidia which have great morphological similarity but different life histories or perfect stages.

The results of the investigations of *M. sitophila* reported here show that the conidial forms of several species of Ascomycetes have heretofore been included under this name. As will be seen from an examination of the descriptions which follow, the differences in the conidia of the different species are comparatively slight, and can be determined only by careful cultural studies made in connection with the perithecial stages. According to our present knowledge the synonomy of *M. sitophila* (Mont.) Sacc. is as follows: *Penicillium sitophilum* Mont.; *Oidium aurantiacum* Lév.; *Monilia martini* Ell. and Sacc.; *Oospora aurantiaca* (Lév.) Herter; *Monilia aurantiaca* (Lév.) Herter not Peck and Saccardo.; *Oidium lupuli* Matth. and Lott. (*31, p. 86–87*); *Oospora lupuli* (Matth. and Lott.) Lindau (*30*); *Monilia aurea* Kitasima not Gmelin; (?) *Monilia carbonaria* Cke.

As the result of the life history and morphological studies of the red bread-mold fungus and its near relatives a new genus, Neurospora, and three new species are described.

Neurospora gen. nov.

Perithecia gregarious or scattered, smooth or with loose hairs, subcoriaceous to subcarbonous; ostiole papillate or short rostrate, perithecial cavity lysigenic, filled at first with parallel, septate hyphae (paraphyses?) which begin to collapse and disappear as soon as the young asci start and are entirely gone when the asci

are full grown (pl. 2, E); spores hyaline to yellowish-brown at first, becoming black or greenish black when mature, continuous, longitudinally ribbed (pl. 3, A). Conidial stage of the *Monilia sitophila* type (pl. 2, B).

The perithecia are either superficial or embedded when grown on agar media; when superficial they usually show a loose growth of weak hyphae on the surface. The crowded septate hyphae which fill the perithecial cavity at the time the asci start are doubtfully described as paraphyses. They disappear so soon that they would not be observed except in very young perithecia.

Neurospora sitophila sp. nov.

Conidial stage (*Monilia sitophila* Mont.): On corn-meal agar tufted or irregularly pulvinate at first, soon becoming effuse and fluffy; conidial masses varying from pale salmon to orange color; mycelium hyaline, creeping, septate; sporogenous hyphae irregularly ascending, septate, dichotomously branched; conidia catenulate, connected by a narrow isthmus when mature, globose to subglobose, smooth, pale salmon to orange in mass, 10 to 12μ diameter, mostly 10 to 11μ (pl. 2, B).

Perithecial stage: Perithecia gregarious or scattered, superficial or immersed on agar media, smooth or loosely soft, hairy, brown to black subcoriaceous, 200 to 300μ diameter; ostiole papillate; asci cylindrical, short stipitate, apex with a thickened gelatinous ring, 8-spored, 140–160$\times$12–14μ paraphyses (?) crowded, multiseptate, filling the perithecium at first, collapsing and disappearing as the asci develop; ascospores overlapping uniseriate, elliptical, with 16 to 17 sometimes branched longitudinal ribs, olivacious becoming dark greenish black, 20–26$\times$10–15μ, mostly 23–25$\times$13–15μ (pl. 4, A; pl. 3, A).

Type: Selected, culture No. 5574, slide No. 5575, from which Plate 4, A, was taken and in mycological collections, Bureau of Plant Industry. Original from Herter, Berlin.

The description is based on cultures on corn-meal agar in test tubes. Whether this species is identical with Montagne's fungus it is impossible to determine at present, as the ascospore stage of his plant was not found. This is the common red bread mold of Europe and America and presumably Montagne's (*35*) species; for practical purposes, therefore, this name may be adopted.

Neurospora crassa sp. nov.

Conidial stage (*Monilia crassa* nom. nov.): On corn-meal agar tufted or irregularly pulvinate at first, soon becoming effuse and forming fluffy aerial masses at the sides of the tube, pale salmon to light orange. Mycelium scanty, creeping, septate; sporogenous hyphae, irregular, ascending, septate, dichtomously branched; conidia catenulate, connected by a narrow isthmus, globose to subglobose when mature, smooth, yellow to pale orange in mass, 6 to 8μ diameter, mostly 6 to 7μ (pl. 2, C).

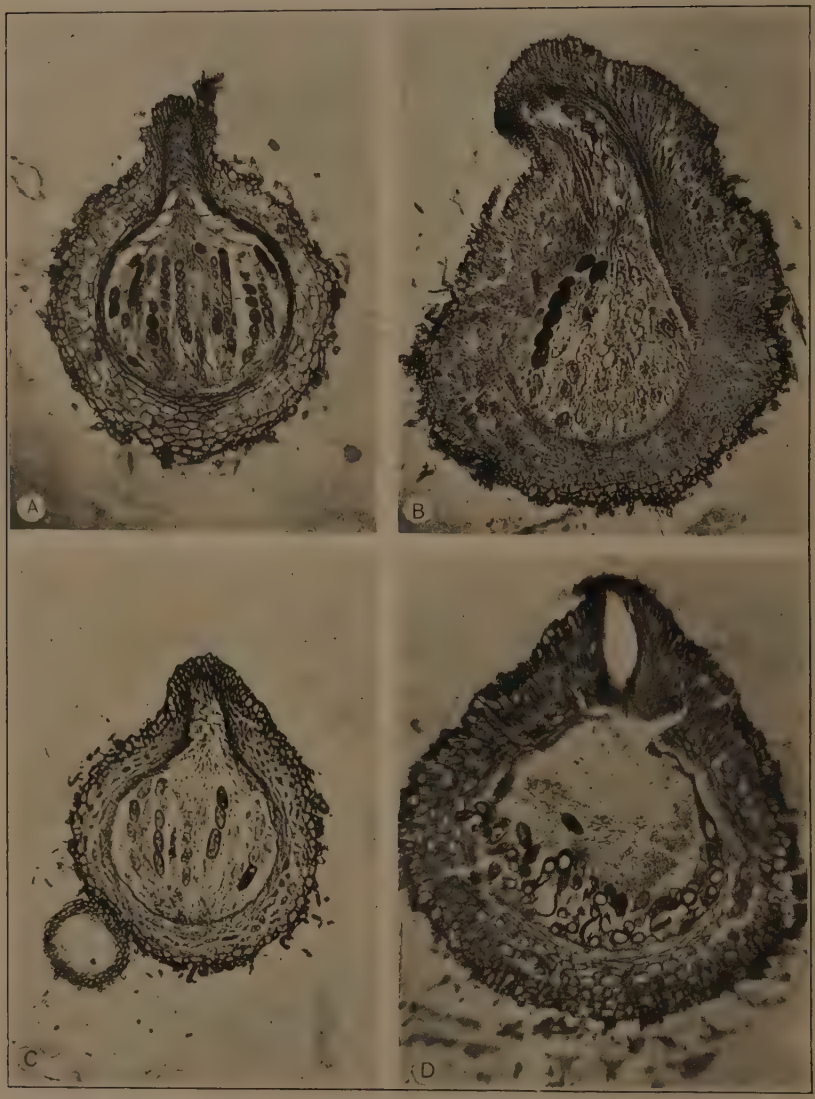
Perithecial stage: Perithecia gregarious or scattered, subglobose, superficial or immersed when grown on agar, smooth or loosely soft hairy when superficial, yellowish brown at first, becoming blackish brown, thick walled, subcoriaceous, 400 to 600μ diameter; ostiole obtuse, conical, black; asci cylindrical, short stipitate, with a thick gelatinous ring at the apex, 4-spored, 150–175$\times$18–20μ; paraphyses (?) crowded, multiseptate, filling the perithecium at the time the asci appear, collapsing and disappearing as the asci develop, entirely lacking in the mature perithecium; ascospores overlapping-uniseriate, elliptical, with about 20 longitudinal, sometimes branched ridges, olivaceous at first, becoming dark brown to black, 23–32$\times$11–16μ, mostly 27–30$\times$14–15μ (pl. 4, B; pl. 3, B). This species is heterothallic.

Habitat: On bagasse, Marrero, La. From Charles Thom, of the Bureau of Chemistry. Known only from this material.

Type: Culture No. 5576, slide No. 5577, from which Plate 4, B, was taken. In mycological collections, Bureau of Plant Industry.

On corn-meal agar in Petri dishes the conidial masses form about the sides of the dish and are about the same color as in *Monilia sitophila*, but smaller in quantity than in that species when grown on the same medium and under the same conditions. The same difference occurs in the cultures but is less striking. On bread in flasks the conidial growths are very similar.

Photomicrograph of asci and ascospores of Neurospora and Melanospora: A. *N. sitophila;* B, *N. crassa;* C, *N. tetrasperma;* D, *Melanospora destruens* × 332



Photomicrographs of sections of perithecia of four species of Neurospora: A, *N. sitophila;* B., *N. crassa;* C, *N. tetrasperma;* D, *N. erythraea.* ×126

Neurospora tetrasperma sp. nov.

Conidial stage (*Monilia tetrasperma* nom. nov.): On corn-meal agar in tubes very scanty whitish growth of mycelia about the upper edge of the agar slant; conidial tufts usually small and scanty, pale salmon; sporogenous hyphae irregularly ascending, septate, dichotomously branched; conidia catenulate, connected by a narrow isthmus when mature, globose to subglobose, smooth, nearly hyaline or pale salmon in mass, 8–11μ in diameter, mostly 8–9μ (pl. 2, D).

Perithecial stage: Perithecia gregarious or scattered, superficial or immersed when grown on agar media, smooth or loosely and sparsely soft hairy when superficial, yellowish when young, becoming dark brown to black; 250–300μ in diameter; wall medium thick, subcoriacious; ostiole obtuse, papillate; paraphyses (?) crowded, septate, collapsing and disappearing as soon as the asci appear; ascospores uniseriate or somewhat overlapping, oblong-elliptical with about 20 longitudinal, sometimes branched ridges, olivaceous at first, becoming dark brown to black, 29–35×14–16μ, mostly 30–31×15μ (pl. 4, C; pl. 3, C). This species is homothallic.

Habitat: Substratum unknown, Surinam, Dutch Guiana, Stahel.

Type: Culture No. 5578, slide No. 5579 from which plate 4, C, was taken. In mycological collections, Bureau of Plant Industry.

On bread the conidia are formed in abundance and have the same general character and appearance as *M. sitophila*.

The cultures of this came originally from Gerald Stahel, Government mycologist, Parimaribo, Dutch Guiana, and were sent the writers from the Central bureau voor Schimmelcultures at Baarn, Holland. These cultures when received contained perithecia.

Neurospora erythraea (Möll.) comb. nov.

Conidial stage (*Monilia erythraea* comb. nov.): Tufts abundant bright orange color in mass; fertile hyphae dichotomously branched, conidia catenulate globose to subglobose, smooth, 7 to 11μ diameter, mostly 8 to 9μ (pl. 2, A).

Perithecial stage: Perithecia gregarious, superficial subglobose loosely and sparsely soft hairy, yellow at first becoming reddish and finally dark brown, thick walled, subcoriacious, 400 to 600μ diameter; ostiole obtuse, short conical; asci cylindrical, stipitate, gelatinous ring at apex, 4-spored, about 250μ long; paraphyses lacking in mature perithecia; ascospores overlapping uniseriate, elliptical with 20 to 22μ longitudinal somewhat branched ridges, yellowish at first, becoming greenish black to black, 28–38×13–18μ, mostly 34–36×15–16μ. This species is apparently homothallic (pl. 4, D).

Habitat: On corn bread and burned stumps of "Rocas" Blumenau, Brazil, A. Möller (*34, p. 294*).

Type: No. 190 (5580). Slide No. 5581. From Möller's collection in alcohol in Botanical Museum, Berlin. In mycological collections, Bureau of Plant Industry.

The above description and Plate 4, D, were made from a part of Möller's original culture sent by H. W. Wollenweber of Berlin, which shows only the perithecia with 4-spored asci. Möller also mentioned finding perithecia with 8-spored asci associated with similar conidia. The 8-spored form was found on a burned stump of orange and was thought by him to be merely a form of *Neurospora erythraea*. It is more probable, however, that this was a distinct species.

Having no living material of Möller's plant, the writers can not prove that it was homothallic. The fact that he obtained perithecia in cultures made by the transfer of a bit of hyphae and that the writers' 4-spored species is homothallic would indicate the probability that such is the case in *Neurospora erythraea*. This species is distinguished from *N. tetrasperma* by its much larger perithecia and ascospores.

HETEROTHALLISM IN THE ASCOMYCETES

The discovery of heterothallism in the Mucorineae by Blakeslee (*4*) and the work on heterothallism in the higher basidiomycetes inaugurated by Mlle. Bensaude (*1*) and Kniep (*26*) need not be reviewed

here. Proof that species of ascomycetes are also heterothallic has been produced in but few cases. The work of Edgerton (*14*) has been frequently cited as showing that *Glomerella cingulata* is heterothallic. Edgerton makes no such claim in his paper. He distinctly states that both the strains produced perithecia when grown alone. However, when the same two strains were grown together on opposite sides of a Petri dish culture more perithecia were produced along the line where the mycelia met than when the strains were grown separately. This is a well known phenomenon. Many instances have been cited in the literature showing that bacteria or other fungous contaminations as well as different colonies of the same fungus often have a beneficial effect in changing the constitution of the medium or in otherwise stimulating the production of ascocarps. The erroneous idea seems to prevail that when fruiting bodies are produced along a line where two mycelia meet this is an indication of heterothallism.

Ascobolus magnificus was the first ascomycete to be definitely proved by the combination of monosporous cultures to be heterothallic (*13*). Only certain pairs of such cultures gave rise to ascocarps. Monosporous mycelia always remained sterile. This species produces functional ascogonia and antheridia which appear to rise from different hyphae. No sex organs are produced in monosporous cultures. In all probability the gametophytes come to maturity and produce their reproductive structures only when grown together in the same medium, the mycelium of each sex so changing the nature of the medium as to stimulate the development of reproductive structures on the mycelium of the opposite sex.

Kirby (*23*) has reported that *Ophiobolus graminis* is heterothallic, ascocarps being produced only when cultures are grown together in certain combinations, monosporous cultures remaining sterile. Kirby believes that the fertile strains are sexually differentiated. Davis (*8*), working with monosporous cultures of the same fungus originally isolated by Kirby, finds that ascocarps are developed in abundance from monosporous cultures. He has repeatedly isolated other single spore cultures of the same species which likewise developed ascocarps. Davis finds that strains from Arkansas and Kansas could not be induced to sporulate. Kirby in recent correspondence with the writers as a result of further work has modified his published statements regarding the heterothallism of this fungus. In view of the fact that Davis states that this Ophiobolus is homothallic and not heterothallic as claimed by Kirby, it is clear that further study on this species is necessary before the question can be considered settled.

Derx (*11*) working with species of Penicillium has proved conclusively that *Penicillium luteum* is heterothallic. Several single ascospore cultures were isolated and grown in all possible combinations. He found that about half the ascospores were of one sex and half the other.

Betts (*3*) has proved beyond a doubt that *Ascobolus carbonarius* is also heterothallic. He grew cultures from seven spores from a single ascus and found that three of them belonged to one sex and four to the other.

CULTURE STUDIES

The material used for cultures has been obtained from different countries, as noted in part in connection with the discussion of the three species of Neurospora described above. This material turned out to be of two different kinds. In all cases each culture had developed the Monilia stage more or less luxuriantly at the time it was received. A few of the cultures had developed ascocarps in transit; one culture produced perithecia later when transferred to a more suitable medium. Each collection was at first designated either by a name, number, or source without particular regard to its specific identity, which was in most cases not determined until later, as will be explained in connection with Table 6. It may be said here that with the exception of Nos. 1, 2, and 15, all of these cultures were later referred to *Neurospora sitophila*. A list of the cultures used, together with their history as far as known to the writers at the time this work was done, follows:

1. *Neurospora crassa*. The material for the work with this form was obtained from Charles Thom. It has been made the type of *N. crassa*. It was originally obtained in 1924 from sugar-cane bagasse near New Orleans.

2. S 227. A culture sent in by J. E. Flynn, Cornell University, who obtained it from H. H. Whetzel.

3. Herter. This material was obtained from the Centraalbureau voor Schimmelcultures at Baarn, Holland. It came originally from Herter and is presumed to be the same material studied by him. It has been made the type of *Neurospora sitophila*. The culture developed mature ascocarps and an abundance of conidia.

4. Japanese. The culture containing ascocarps and conidia received from Doctor Kitasima, Tokyo, Japan. The culture was labeled "single ascospore."

5. Arlington. The culture of the fungus which appeared as a contamination in Petri dish cultures in the greenhouse at Arlington Experiment Farm, Rosslyn, Va. After transfers to corn-meal agar in test tubes this material developed ascocarps as well as conidia.

6. B. P. I. A culture obtained from a contamination in a Florence flask containing fruit juice in a laboratory at the Department of Agriculture, Washington, D. C.; appeared about the same time as No. 5, the Arlington culture, and may have been originally from the same source. Ascocarps and conidia developed on the fruit juice in the flask.

7. Scales. A culture originally sent by F. M. Scales, of Mount Vernon, N. Y., obtained by him from a bakery in New York City. The culture as originally received produced a great abundance of conidia. A large number of subcultures obtained by transfer from the original material to corn-meal agar developed ascocarps.

8. Bainier. A culture obtained from Thom, as representing the Bainier material kept in culture in l'Ecole de Pharmacie, at Paris. This culture developed conidia and numbers of small sclerotiumlike bodies, but no ascocarps.

9. B 704. Culture obtained from the laboratory of H. H. Whetzel, who had originally received it from Wooster, Ohio, produced only conidia.

10. Rose. A culture which developed only conidia. Contributed by D. H. Rose, who had obtained the fungus from rotting strawberries at Chadbourn, N. C.

11. Columbia University. A culture from Columbia University. According to the history of the culture it was originally obtained from a bakery in St. Louis, Mo., by Kunkel (*28*).

12. Berkhout. A culture which developed conidia and numbers of very small bulbils or sclerotia was a part of the material reported on by Christine Berkhout (*2*).

13. Kauffman. Material contributed by C. H. Kauffman, Ann Arbor, Mich., produced only conidia.

14. Cooley. A culture obtained by J. S. Cooley from an apple-receiving room in a cold-storage plant at Charlottesville, Va. The same fungus had been obtained repeatedly from the air in apple-packing sheds.

15. *Neurospora tetrasperma*. A culture obtained from the Centralbureau voor Schimmelcultures at Baarn, Holland. The culture was originally contributed

by G. Stahel from Surinam. The culture had developed large numbers of ascocarps when received and only a very few conidia. Like *N. erythraea*, the species described by Möller (*34, p. 294*), each ascus develops only four spores.

SPORE GERMINATION

The conidia of the Monilia stage germinate readily under all ordinary cultural conditions. According to the statements of Tokugawa and Emoto (*48*) and others, the conidia when in a fairly dry condition resist for 15 or 20 minutes temperatures considerably above the boiling point of water.

The writers have found that in case of the *Monilia sitophila* there are a number of factors that must be considered in determining the effect of heat on germination. When the conidia are thoroughly wet most of them are killed when heated for five minutes at 65° C. and all are killed when kept at 72° C. for the same time. Should a little air be caught in a tangle of conidia and fragments of hyphae, some of the conidia will not be wet even though immersed, and these will survive when heated at much higher temperatures. It has also been found that, as reported by Thom and Ayers (*46*), heating of the conidia delays their germination sometimes for several days, so that the observations should be made for a period of at least 10 days after the experiment has been made.

In this connection Thom and Ayers in studying the effect of Pasteurization on mold spores found that some of the conidia of *Aspergillus fumigatus*, for example, germinated after having been floated on the surface of milk in a test tube and heated for 30 minutes at 65.6°C. These authors found that nearly all of the conidia of the different molds tested were killed when immersed in milk at 68.3° for 30 seconds.

The question as to whether or not conidia in flour survive the baking process would depend on whether they are thoroughly wet in mixing the dough, how porous the dough is after rising, and whether they start to germinate before being heated. The size of the loaf, the temperature of the oven, the length of time the loaf is baked, and the rapidity with which the loaf is cooled are some of the factors that must be taken into account.

Another important factor not heretofore considered is the presence of ascospores of the fungus. As will be pointed out later, the ascospores are much more resistant to heating under moist conditions than are the conidia. Experiments are being carried out in cooperation with L. H. Bailey of the Bureau of Chemistry to determine more definitely the effect of various temperatures and conditions of baking upon the viability of spores contained in the dough.

For the study of heterothallism the conidia alone could not serve as the primary source of pedigreed cultures. The ascospores rarely germinate under ordinary culture conditions. Möller (*34, p. 294*) was unable to obtain germination of ascospores of *Neurospora erythraea*. Kitasima (*25*) evidently had great difficulty in securing cultures from ascospores. Any attempt to obtain pedigreed cultures from spores which so rarely germinate under ordinary conditions would be a very tedious process and would often result in contaminations by conidia of the species. In order to secure numerous ascospore cultures it was necessary to find some method of insuring germination of the ascospores.

The method of germinating ascospores of certain species of Ascobolus described by Dodge (*12*) and further tested out by Ramlow (*39*) and Betts (*3*) was tried out and found to be highly satisfactory. The ascospores are sown on the surface of an agar medium in a Petri dish and spread out so that the spores may be picked out separately later. The Petri dish is then set in any ordinary drying oven where the temperature can be raised gradually from room temperature to 90° C. The thermostat may be so regulated as to require 20 or 30 minutes to do this. The exact temperature to which the spores are raised is not of vital importance. It will be found that a large percentage of ascospores so treated will begin to germinate within four or five hours. Transfers of single germinating spores must be made within a few hours, since the germ tubes grow rapidly and tend to become interlaced with those from other spores. A spore usually germinates from a pore in each end. A rather large globule is first extruded and from this vesicle usually two germ tubes develop and grow out in opposite directions (pl. 1, C–F).

When quantities of conidia are carried over with the ascospores in making the sowings, it is advisable to allow the cultures to stand a few hours so that the conidia may begin to germinate. The heating necessary to stimulate ascospore germination will kill the germinating conidia and facilitate the isolation of single ascospores. A better way is to use only material in which the perithecia are beginning to discharge the spores, which are shot some distance. The bottom of a Petri dish containing agar may be inverted over the culture bearing the perithecia so as to catch the ascospores as they are shot from the fruit bodies. In this way very few conidia will be carried up to the agar and the ascospores will be scattered far enough apart on the surface of the agar to be removed separately with little difficulty after germination. The percentage of germination is ordinarily high when the spores have been properly heated. The higher the temperature within the limits given above the more satisfactory will be the results of attempts to obtain cultures free from conidial contamination.

It has been found that corn-meal agar is an excellent medium for this culture work. Conidia are not produced in such abundance on this medium as on some others. While the three species studied produce perithecia under proper conditions when grown on corn-meal agar in Petri dishes, it is much better to use tube cultures. It goes without saying that in working with a fungus which produces conidia in such masses certain precautions are necessary to avoid contaminations, especially when material from many different sources is to be handled.

NEUROSPORA CRASSA

Following the culture methods described above, over 100 single ascospore cultures have been obtained. These were supplemented by several single conidium cultures, and mixed cultures derived from several ascospores were also made for comparison.

Svedelius (*45*) and others are now using the term "haplont" to distinguish a particular plant of the gametophytic phase of an alga or fungus. In the case of such a fungus as Neurospora, the gametophytic growth originating from a single ascospore is capable of

reproducing itself indefinitely by fragments of the hyphae or by the formation of conidia. The mycelium or haplont derived in this way would be analogous merely, but not homologous, to a clon in the higher plants. While no one is now misled by such statements as growing, pairing, or mating cultures or strains in different combinations, it is simpler in this connection to refer to monosporous cultures as haplonts.

The monosporous mycelia (haplonts) at first produced only conidia. After about 10 days some of them began to form structures which in size and color look like young perithecia. Such sclerotium-like bodies resembling the bulbils of certain species of the form genus Papulospora have been noted by several persons who have worked with *Monilia sitophila*, as already mentioned. These bodies were observed for several months but none developed ascospores. Several hundred subcultures were made by transferring the tips of hyphae or by transferring conidia; no perithecia were produced in these cultures. The mixed cultures, however, which were derived from three or four ascospores, usually developed perithecia within two weeks.

The cultures originally derived from single ascospores have been grown in different combinations. The results obtained by pairing 15 different haplonts in all possible ways, and in duplicate, are tabulated so that the cultures fall into two groups. (See Table 1.) The marginal and top numbers are used to designate particular cultures. Failure to produce perithecia is indicated by the minus sign; a positive sexual reaction and the formation of normal perithecia is indicated by the plus sign. The table shows that any mycelium in one group when grown with any other mycelium of the same group produces only conidia; when a mycelium from one group is grown with any mycelium from the other group perfect perithecia as well as conidia are matured. The results of this work prove conclusively that *Neurospora crassa* is heterothallic.

TABLE 1.—*Monosporous mycelia or haplonts, from random selection of ascospores of Neurospora crassa; haplonts paired in all possible ways and in duplicate* [a]

	1	4	5	8	16	19	2	3	6	7	9	10	11	12	20
1	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+
4	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+
5	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+
8	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+
16	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+
19	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+
2	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−
3	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−
6	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−
7	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−
9	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−
10	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−
11	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−
12	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−
20	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−

[a] Marginal and top numbers refer to particular cultures. Failure to produce perithecia indicated by −. A positive sexual reaction and the formation of normal perithecia is indicated by +.

Each ascus of this species contains eight spores which are shot violently from the ascus at maturity. Occasionally the eight spores so discharged fall in a separate group. They can then be removed separately and germinated. Five sets of such haplonts, each set

consisting of eight monosporous mycelia derived from spores from the same ascus, were made. The haplonts from each set were then grown in all possible combinations. The results obtained with each of the five sets agree. Table 2 shows the results of mating the mycelia in one set. The marginal and top numbers represent the haplonts from single spores picked up at random after they had been discharged from a single ascus. The results show clearly that four spores from an ascus belong to one sex and four to the other.

TABLE 2.—*Eight monosporous mycelia or haplonts of Neurospora crassa. Spores from the same ascus, picked up at random after their discharge. All combinations* [a]

	1	2	6	7	3	4	5	8
1	−	−	−	−	+	+	+	+
2	−	−	−	−	+	+	+	+
6	−	−	−	−	+	+	+	+
7	−	−	−	−	+	+	+	+
3	+	+	+	+	−	−	−	−
4	+	+	+	+	−	−	−	−
5	+	+	+	+	−	−	−	−
8	+	+	+	+	−	−	−	−

[a] Marginal and top numbers refer to particular cultures. Failure to produce perithecia indicated by −. A positive sexual reaction and the formation of normal perithecia is indicated by +.

NEUROSPORA SITOPHILA

Ascocarpic material of *Neurospora sitophila*, as typified by the Herter material referred to above, has been received from five different sources. The first and most extensive culture work with this species was done with material collected at Arlington, Va. Ninety-six cultures were obtained by germinating single ascospores. These mycelia were grown in several thousand cultures on different media, singly and in different combinations. Monosporous mycelia produce only conidia and in most cases numerous sclerotiumlike bodies. The 96 mycelia were not grown in all possible combinations, but it was proved conclusively that the Arlington material is heterothallic. The results obtained by later cultures of the Herter and Japanese material, which are the only results presented here in tabulated form, substantiate those previously obtained.

A number of haplonts derived from single ascospores of the Herter material have been grown for several months, both alone and in all possible combinations. The results obtained by growing in all possible combinations eight of such mycelia chosen at random are shown in Table 3. The cultures used may be arranged in two groups. Perithecia are produced only when a mycelium from one of the groups is grown together with a mycelium from the other group. For example, mycelium 4 develops only conidia when grown alone or in combination with either mycelium 2 or 6. When, however, haplont 4 is grown with either mycelium 1, 3, 5, or 8, both conidia and perithecia are developed. The Herter material of *Neurospora sitophila* is also heterothallic.

The culture of the Japanese material of *Neurospora sitophila* was maturing perithecia when received. Twenty haplonts or monosporous cultures from ascospores were obtained and subcultures from these

have been grown for nearly a year. In no case has such a culture produced perithecia. Eleven of these haplonts were grown in combination, as shown in Table 3. This shows that the Japanese material of *N. sitophila* is likewise heterothallic. The haplonts fall into two groups. Any mycelium from one group will produce perithecia when grown with a mycelium from the other group. Even though all the cultures of one group be grown together no perithecia are formed.

TABLE 3.—*Monosporous mycelia (haplonts) obtained by random selection of ascospores of Neurospora sitophila (from Herter). All combinations* [a]

	2	4	6	1	3	5	7	8
2	−	−	−	+	+	+	+	+
4	−	−	−	+	+	+	+	+
6	−	−	−	+	+	+	+	+
1	+	+	+	−	−	−	−	−
3	+	+	+	−	−	−	−	−
5	+	+	+	−	−	−	−	−
7	+	+	+	−	−	−	−	−
8	+	+	+	−	−	−	−	−

[a] Marginal and top numbers refer to particular cultures. Failure to produce perithecia indicated by −. A positive sexual reaction and the formation of normal perithecial s indicated by +.

The original culture from Doctor Kitasima was according to the label, obtained from a single ascospore. Without denying that a culture from a single ascospore of this material might for some very definite reason develop ascocarps, the writers merely wish to state here that all of their single-spore cultures from this material produce only conidia and sclerotiumlike bodies when grown alone. Table 4 shows the results of mating haplonts from these cultures.

It is unnecessary to describe in detail the results obtained by growing large numbers of monosporous cultures of this species from ascosporic material received from the other three sources mentioned. The results prove beyond question that the species *Neurospora sitophila* is heterothallic and that single-spore cultures so far obtained have not produced perithecia.

As stated above, the original material from each of the five sources contained both of the elements necessary for sexual reproduction of the species. Frequently, however, mycologists find that cultures of *Monilia sitophila* obtained from rotting fruit, from bakeries, or from burned trees produce only conidia, regardless of what culture medium is employed. A number of such cultures of the Monilia stage have been received within the present year. In some cases hundreds of subcultures were grown in attempts to obtain ascocarps, but without success. It became a matter of much interest to determine whether perithecia could be obtained by mating two or more of these forms together in a culture. The results of this work, discussed further in connection with Table 6, show that cultures may be classed in two groups, based on their sexual reactions. All but one of the conidial cultures received may be referred to the species *Neurospora sitophila* or a species so similar to it morphologically as to be indistinguishable from it.

TABLE 4.—*Monosporous cultures, obtained by random selection of ascospores of Neurospora sitophila (Japanese). All combinations* [a]

	1	3	5	8	9	12	2	4	6	10	11
1	−	−	−	−	−	−	+	+	+	+	+
3	−	−	−	−	−	−	+	+	+	+	+
5	−	−	−	−	−	−	+	+	+	+	+
8	−	−	−	−	−	−	+	+	+	+	+
9	−	−	−	−	−	−	+	+	+	+	+
12	−	−	−	−	−	−	+	+	+	+	+
2	+	+	+	+	+	+	−	−	−	−	−
4	+	+	+	+	+	+	−	−	−	−	−
6	+	+	+	+	+	+	−	−	−	−	−
10	+	+	+	+	+	+	−	−	−	−	−
11	+	+	+	+	+	+	−	−	−	−	−

[a] Marginal and top numbers refer to particular cultures. Failure to produce perithecia indicated by −. A positive sexual reaction and the formation of normal perithecia is indicated by +.

It may be of interest to know, for example, that *Monilia sitophila* obtained from rotting strawberries at Chadbourn, N. C., forms perithecia when grown together with the *M. sitophila* with which Berkhout (*2*) worked in Holland. Both collections produce only conidia when grown separately. The culture from a St. Louis bakery (*28*) and the one from the Bainier collection do not develop perithecia when grown separately or together, but each develops perithecia when paired either with the *M. sitophila* received from a laboratory at Ann Harbor, Mich., or with a culture sent in by Bunzel from a bakery in New York City.

NEUROSPORA TETRASPERMA

As already stated, the culture originally obtained from Doctor Stahel from Surinam had matured large numbers of perithecia when received. The species is characterized especially by the fact that the asci, with few exceptions, contain only four spores. The species produces very few conidia in culture on corn-meal agar, but when grown on bread or such a medium as potato-dextrose agar great quantitites of conidia are formed, so that in the conidial stage it would not be readily distinguished from the red-mold stage of *Neurospora sitophila*. Growing the two species side by side on corn-meal agar, however, would enable one to distinguish readily the Surinam species, which has been described above under the name *N. tetrasperma*.

A number of monosporous cultures were obtained by isolating single ascospores from the heavy spore print formed on the tube of the original culture. Within six or seven days each of these cultures showed mature ascocarps which were discharging spores. This proved conculsively that *Neurospora tetrasperma* is commonly homothallic.

Over 200 additional monosporous mycelia obtained from other ascosporic subcultures of this species have developed perithecia on corn-meal agar.

Möller (*34, p. 294*) noted that in *Neurospora erythraea* the ascospores varied considerably in size. The average size, he stated, was about 36μ long. As observed previously under the discussion of this species, it has been found that the ascospores of the Surinam material, *N. tetrasperma*, average about 30 to 32μ long. These measurements apply to spores from an ascus bearing 4 spores. Careful examinations

of crushed preparations show that occasionally an ascus develops 5 or 6 spores, but more rarely 3, 2, or only 1 spore. In case an ascus develops 5 spores 2 of them will be very small, while the other 3 will be of normal size. An ascus with three spores ordinarily will have 1 spore which is about 40 to 45μ long. One-spored asci are very rare. In the 2 or 3 that have been found the spores were from 60 to 65μ long. The smallest spores are about 20 to 25μ long. Over 50 monosporous cultures of the abnormally large spores have been made. One has no difficulty in distinguishing these large spores from the common type. With the exception of two somewhat doubtful cases which need further study, the results show that each of the unusually large spores possesses all the elements necesary for sexual reproduction and produces good perithecia.

In the case of the very small spores it has been found that the size varies considerably so that it is not always possible to distinguish these small spores definitely from the others. Forty-four monosporous cultures from spores which were judged to be under size were isolated. Only five of these cultures developed perithecia. The others developed large numbers of very small sclerotiumlike bodies and a few conidia. The cultures that remained sterile were then grown in all possible combinations. The results of growing 21 of these haplonts together is shown in Table 5. It will be seen that the 21 cultures fall into two groups. There are 9 cultures in one group and 12 in the other. Mycelium from one group produces perithecia only when grown in combination with mycelium from the second group. In other words, this species which is ordinarily homothallic occasionally develops spores which are not totipotent. Each spore contains only one of the sex elements. By selecting at random the small spores, haplonts may be obtained which when grown in combination develop normal perithecia with asci which produce predominantly only four spores. The results of all the culture work with this species show that regardless of whether perithecia are obtained by growing what may be called the normal spores, or by growing those that are especially large or very small, the resulting perithecia and ascospores are of the same type.

TABLE 5.—*Small spore selections of Neurospora tetrasperma (commonly homothallic) which are heterothallic* [a]

	1	3	D	8	30	32	E	H	J	2	4	6	7	10	28	29	31	33	34	G	5
1	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+
3	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+
D	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+
8	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+
30	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+
32	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+
E	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+
H	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+
J	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+
2	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
4	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
6	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
7	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
10	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
28	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
29	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
31	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
33	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
34	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
G	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
5	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−

[a] Marginal and top numbers refer to particular cultures. Failure to produce perithecia indicated by −. a positive sexual reaction and the formation of normal perithecia is indicated by +.

IDENTIFICATION OF CULTURES BEARING ONLY CONIDIA

Certain cultures which developed only conidia of the Monilia stage are listed on page 1029. In their conidial stage the two heterothallic species are not readily distinguished with certainty. The most practical way by which the species affiliations of the sterile cultures could be determined was to grow them in combination with monosporous cultures of known origin. If, for example, a culture of unknown identity be mated with each of two reciprocal haplonts of *Neurospora crassa*, and if in one case perithecia are produced similar to those normally developed by *N. crassa*, such a result would indicate that the unknown culture probably belongs to that species. In Table 6 the reciprocal haplonts of a species or those which when paired will produce perithecia are designated for convenience as A and B without regard to their sex, which is unknown at present. The unknown strains were grown in combination with several different pairs of reciprocal haplonts of each of the two species *N. crassa* and *N. sitophila*. The results shown in Table 6, as previously stated, prove that with one exception each unknown was one or the other sex of *N. sitophila*.

PERITHECIA RESULTING FROM MATING HAPLONTS OF DIFFERENT SPECIES

For purposes of experiments on crossing or hybridization one may consider as heterothallic the small spore selections of the homothallic species, *Neurospora tetrasperma*, discussed in connection with Table 5. Pairs of reciprocal haplonts of each of the three species and all of the sterile cultures of unknown identity noted above were grown in all possible combinations. These results are also included in Table 6. In case no perithecia were produced the result is indicated as before with the minus sign. When the haplonts mate and perithecia result from the combination this is indicated by the plus sign, and the strains crossed are assumed to belong to the species having a perithecium and spores of the same kind. The results of crossing reciprocal strains or haplonts of one species with reciprocal strains of another species are indicated by the minus sign when no sexual reaction occurred, and by the letter X when the reaction results in the formation of ascocarps with mature spores. In these cases the course of development of the perithecia and ascospores differs from that of either species. There are various differences in the form and size of the spores, and the lack of uniformity in reaching maturity is such as would be expected in hybrids.

TABLE 6.—*Haplonts of unknown identity and those known to belong to Neurospora sitophila (from various sources), N. crassa (Thom coll.), and N. tetrasperma (Stahel coll.), paired in all possible combinations* [a]

	N. sitophila from—					Unknowns from—							N. sitophila from—					Unknowns from—					
	Herter A	Japanese A	Arlington A	B. P. I. A	Scales A	Bainier	B 704	Rose	Columbia University	N. crassa A	N. tetrasperma from—tetrasperma A		Herter B	Japanese B	Arlington B	B. P. I. B	Scales B	Berkhout	Kauffman	Cooley	S 227	N. crassa B	N. tetrasperma from—tetrasperma B
N. sitophila:																							
Herter A	−	−	−	−	−	−	−	−	−	−	−		+	+	+	+	+	+	+	+	X	X	X
Japanese A	−	−	−	−	−	−	−	−	−	−	−		+	+	+	+	+	+	+	+	X	X	X
Arlington A	−	−	−	−	−	−	−	−	−	−	−		+	+	+	+	+	+	+	+	X	X	X
B. P. I. A	−	−	−	−	−	−	−	−	−	−	−		+	+	+	+	+	+	+	+	X	X	X
Scales A	−	−	−	−	−	−	−	−	−	−	−		+	+	+	+	+	+	+	+	X	X	X
Unknowns:																							
Bainier	−	−	−	−	−	−	−	−	−	−	−		+	+	+	+	+	+	+	+	X	X	X
B 704	−	−	−	−	−	−	−	−	−	−	−		+	+	+	+	+	+	+	+	X	X	X
Rose	−	−	−	−	−	−	−	−	−	−	−		+	+	+	+	+	+	+	+	X	X	X
Columbia University	−	−	−	−	−	−	−	−	−	−	−		+	+	+	+	+	+	+	+	X	X	X
N. crassa A	−	−	−	−	−	−	−	−	−	−	−		X	X	X	X	X	X	X	X	+	+	X
N. tetrasperma A	−	−	−	−	−	−	−	−	−	−	−		X	X	X	X	X	X	X	X	X	X	+
N. sitophila:																							
Herter B	+	+	+	+	+	+	+	+	+	X	X		−	−	−	−	−	−	−	−	−	−	−
Japanese B	+	+	+	+	+	+	+	+	+	X	X		−	−	−	−	−	−	−	−	−	−	−
Arlington B	+	+	+	+	+	+	+	+	+	X	X		−	−	−	−	−	−	−	−	−	−	−
B. P. I. B	+	+	+	+	+	+	+	+	+	X	X		−	−	−	−	−	−	−	−	−	−	−
Scales B	+	+	+	+	+	+	+	+	+	X	X		−	−	−	−	−	−	−	−	−	−	−
Unknowns:																							
Berkhout	+	+	+	+	+	+	+	+	+	X	X		−	−	−	−	−	−	−	−	−	−	−
Kauffman	+	+	+	+	+	+	+	+	+	X	X		−	−	−	−	−	−	−	−	−	−	−
Cooley	+	+	+	+	+	+	+	+	+	X	X		−	−	−	−	−	−	−	−	−	−	−
S 227	X	X	X	X	X	X	X	X	X	+	X		−	−	−	−	−	−	−	−	−	−	−
N. crassa B	X	X	X	X	X	X	X	X	X	+	X		−	−	−	−	−	−	−	−	−	−	−
N. tetrasperma B	X	X	X	X	X	X	X	X	X	X	+		−	−	−	−	−	−	−	−	−	−	−

[a] In this table A and B are reciprocal as to sex reaction. The minus sign indicates that no perithecia were produced; the plus sign indicates that normal perithecia developed; the letter X indicates that hybrid perithecia with mature asci were formed.

As noted above the table shows that with the exception of S 227 all of the cultures received which were sterile with regard to ascocarps when grown alone, belong to the species *Neurospora sitophila*. The culture S 227 formed perfect perithecia when grown with haplont A of *N. crassa*, so that it is known to belong to the species *N. crassa*, whose perithecium has the same characters. After these results were obtained and the history of the culture further determined it was found that Whetzel originally received the culture from Thom, who had also contributed the material for the culture work conducted by the writers on *N. crassa*. In making the transfers from Thom's cultures which were producing perithecia, only the B strain was sent to Whetzel. At least the culture S 227 with which the writers did their work remained sterile as long as it was cultured alone.

Any haplont A of the species *Neurospora crassa* and a haplont B of *N. sitophila* when mated develop a few large perithecia which

mature a relatively small number of ascospores. A positive reaction occurs when the heterothallic haplonts A of *N. tetrasperma* are mated with haplonts B of either *N. crassa* or *N. sitophila*, resulting in the development of perithecia with ascopores.

At this writing no culture work has been done with spores from perithecia which may be true sexual hybrids between the species; that is, the ascocarps which are developed when strains of different species are crossed may really be produced as the result of unions between reciprocal haplonts. Further work will be necessary before any conclusion can be reached concerning the nature of these structures. The results of cytological work on Neurospora will be published in another connection.

SUMMARY

The red bread-mold fungus, *Monilia sitophila* Mont., was first described in 1843. It has frequently been found seriously infesting bakeries in Europe and America. It has also been reported on various fruits and tree trunks and stumps recently killed by burning, and on sugar-cane bagasse.

This study shows that instead of one species there are at least four which have been passing under this name. The life histories of these four species have been determined.

Instead of belonging to Sclerotinia, as might be expected from the great resemblance of the conidia to those of *Monilia cinerea*, the ascospore stage is found to be a pyrenomycete which seems to belong to an undescribed genus, which is named Neurospora.

The conidia of three species grow readily at ordinary laboratory temperatures. When exposed to a temperature of 120° C. for 30 minutes dry conidia are killed; when wet they are killed when exposed to a temperature of 72° for 5 minutes. The ascospores are more resistant when dry.

The ascospores do not germinate under ordinary cultural conditions, but grow readily after heating in a moist condition at 65° to 70° C. for a few minutes.

Baking tests show that 1-pound loaves in which have been placed both conidia and ascospores and which have been baked in an oven for 35 minutes at 215° C. do not develop mold.

Bakeries contaminated with these fungi can be freed from them by thorough cleaning of walls, floors, and utensils with live steam, soap and water, formaldehyde, or any good antiseptic.

Two of the species, *Neurospora sitophila* and *N. crassa*, have been found to be heterothallic. *N. tetrasperma* is usually homothallic, but occasionally heterothallic haplonts have been obtained by the selection of abnormal spores. *N. erythraea* has not been studied in culture by the writers, but is probably homothallic. Cultures from single spores of the heterothallic species produce only conidia. Reciprocal haplonts when mated produce perithecia.

Crossing the different species by mating their haplonts results in the formation of perithecia with ascospores which seem to have hybrid characters.

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